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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<b>(21) International Application Number:</b> PCT/GB91/00633 <b>(22) International Filing Date:</b> 22 April 1991 (22.04.91)  <b>(30) Priority data:</b> 9009106.7                      23 April 1990 (23.04.90)                      GB  <b>(71) Applicant (for all designated States except US):</b> 3I RESEARCH EXPLOITATION LIMITED [GB/GB]; 91 Waterloo Road, London SE1 8XP (GB).  <b>(72) Inventor; and</b> <b>(75) Inventor/Applicant (for US only) :</b> STEVENSON, George, Telford [AU/GB]; 9 Meadowhead Road, Southampton SO1 7AP (GB).  <b>(74) Agent:</b> GADSDEN, Robert, Edward; 3i Research Exploitation Limited, The Gate House, 2 Park Street, Windsor, Berkshire SL4 1LU (GB).		<b>(81) Designated States:</b> AT (European patent), BE (European patent), CH (European patent), DE (European patent), DK (European patent), ES (European patent), FR (European patent), GB (European patent), GR (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent), US.  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> PROCESSES AND INTERMEDIATES FOR SYNTHETIC ANTIBODY DERIVATIVES  <b>(57) Abstract</b>  Intermediates for use in the preparation of derivatives of Fc regions of antibodies, a process for making such intermediates, use of such intermediates in the manufacture of synthetic antibody derivatives and antibody derivatives produced by such use.		

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PROCESSES AND INTERMEDIATES FOR SYNTHETIC  
ANTIBODY DERIVATIVES

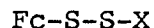
Co-pending, application PCT/GB89/01269 (published as  
5 WO 90/04413) relates to antibody derivatives having two  
or more Fc regions and to processes and intermediates for  
making such derivatives. This invention relates to  
alternative processes and intermediates having advantages  
in certain respects over those described in said earlier  
10 application.

Antibody derivatives with multiple Fc regions have a  
variety of possible therapeutic applications, especially  
when the Fc regions are linked to Fab regions or  
functionally similar moieties to form combinations having  
15 "chimeric" properties.

In order to construct such combinations in a  
controlled way it is advantageous to use, as  
intermediates, Fc regions having a controlled number of  
chemically functional groups which may be reacted with  
20 complementary functional groups on other moieties.

The present invention provides intermediates of this  
type, processes for making them and processes for using  
them in the manufacture of synthetic antibody derivatives.

Accordingly the present invention provides  
25 intermediates for use in the preparation of derivatives  
of Fc regions of antibodies, the said intermediates  
having the general formula:



where Fc is an Fc region in which external sulphydryl  
30 groups have been rendered substantially inert  
by incorporation into protein-protein  
disulphide bonds or by alkylation  
and X is a protonated group causing the disulphide  
linkage to be susceptible to reduction (i.e.  
35 highly electrophilic) at a relatively low pH.

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By "external" we mean those sulphydryl groups released by reduction of interchain disulphide bonds. We distinguish these from sulphydryl groups arising from reduction of intrachain disulphide bonds: these  
5 intrachain bonds are buried internally in the protein, are difficult to reduce without substantially unfolding the protein and remain intact throughout all manipulations referred to in this application.

It will be understood by those skilled in the art  
10 that "inert" is not a completely absolute term. In this context we intend it to mean stable under process conditions to which the intermediate is to be subjected and in conditions of use, thus distinguishing from temporary masking groups and the like. Sulphydryl groups  
15 may, for example, be rendered inert by alkylation.

The group X is preferably pyridyl, permitting reduction at a low pH in the region of 4 where other disulphide bonds, for example inter-chain disulphide links and the like, are not reduced.

20 The term "Fc region" is intended to include not only, for example, typical immunoglobulin Fc structures comprising two peptide chains but also partial structures, active fragments and the like retaining Fc activity, e.g. recruitment effector ability or  
25 involvement in Ig transport and metabolism.

Fc regions may be derived from immunoglobulin of any immunoglobulin class or animal species but, if intended for human use, are preferably from human immunoglobulin, e.g. IgG or IgG1. Polyclonal or monoclonal sources may  
30 be used.

In general terms, intermediates of the present invention may be produced by the following process steps:

- a) combining an Fc region with a "sacrificial" group;
- b) subjecting the combination so formed to disulphide  
35 interchange and rendering potential reactive sites inert;
- c) separating the sacrificial group from the Fc region

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to leave a reactive site on the Fc region; and  
d) attaching a protonated group to the reactive site.

The sacrificial group is preferably selected from groups of sufficient molecular weight to enable  
5 separation of mono-substituted Fc regions from other reaction products by chromatographic fractionation or the like. One suitable group, for example, is an Fab' region from sheep immunoglobulin. This has the advantage of being a cheap and readily available source of  
10 immunoglobulin providing an Fab'<sub>2</sub> fragment with a single inter-gamma disulphide bond which can be cleaved to yield Fab'-SH and then Fab'-SS-Py with a single pyridyl disulphide. Another suitable group is human serum albumin.

15 Preferably the protonated group is pyridyl which may be attached to a reactive SH group by reacting with dipyridyl disulphide.

A more precise illustration of the method of the invention is the preparation of Fc $\gamma$ -dithiopyridine  
20 (pyridyl-Fc, Fc-SS-Py), a stable derivative of human normal IgG1 with a single pyridyl group protruding from a cysteine residue in the Fc $\gamma$  hinge. When required for use Fc-SS-Py is quickly converted to its active form Fc $\gamma$ -monomaleimide (Fc-mal), in which the hinge cysteine  
25 bears a metastable maleimidyl group. This group enables the Fc $\gamma$  to be linked to any protein with an accessible SH group, yielding chimeric derivatives joined by thioether bonds.

This preparation begins with papain digestion of  
30 human normal IgG1 to yield Fc $\gamma$ . An initial reduction of the Fc $\gamma$  hinge releases four SH groups from the two parallel inter- $\gamma$  SS bonds. One of these groups is protected by temporary incorporation into an SS link to a disposable protein (sacrificial group) (human albumin or  
35 sheep Fab' $\gamma$ ). The remaining three SH then undergo disulphide interchange with 4,4'-dipyridyl disulphide (Py-SS-Py), reconstituting an inter- $\gamma$  SS from the paired

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SH groups and forming the mixed disulphide protein-SS-Py from the unpaired SH. This protein product is now exposed to dithiothreitol at pH 4.0 - where the inter- $\gamma$  and Fc $\gamma$ -albumin (or Fc $\gamma$ -Fab' $\gamma$ ) SS bonds are not  
5 susceptible to reduction, but the bond in protein-SS-Py (highly electrophilic because of protonation of the pyridyl N) is. A solitary hinge SH results, and is then permanently blocked by alkylation with N-ethylmaleimide. The Fc $\gamma$ -albumin chimera is purified chromatographically,  
10 cleaved by reduction of all interchain SS, and the albumin is discarded. The Fc $\gamma$  with three hinge SH now undergoes a second round of disulphide interchange with Py-SS-Py to form the final Fc-SS-Py product, with a reconstituted hinge SS and a mixed disulphide  
15 (protein-SS-Py) formed from the unpaired SH.

The preparation may be simplified by omitting the first round of disulphide interchange with Py-SS-Py, proceeding instead to alkylate all three hinge SH after formation of the Fc $\gamma$ -albumin chimera. The final  
20 Fc-SS-Py product then has an open hinge, which may be advantageous in certain situations (for example in not activating complement).

When required for use Fc-SS-Py is reduced with dithiothreitol at pH 4.0 to leave a solitary protein SH  
25 which, upon reaction with a molar surplus of a bismaleimide R-(mal)<sub>2</sub>, forms Fc $\gamma$ -S-succinimidyl-R-maleimide, i.e. Fc-mal.

The invention makes possible the production of a range of intermediate and final product structures and a  
30 variety of process steps and combinations. Such further possibilities will be readily understood by those skilled in the art and the present invention includes within its scope novel processes, intermediates and products derived from the invention whether as individual features or in  
35 combination with each other or further features to produce novel combinations.

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Various aspects of the invention will now be exemplified in more detail with reference to the drawings in which:

- Figure 1 illustrates the preparation of a precursor to the intermediate of the invention, of the form Fab-SS-Fc, in which a sacrificial Fab is combined with an Fc region having a sulphydryl group which has been rendered inert by alkylation (represented in the drawings by the symbol S●);
- Figure 2 illustrates a subsequent stage in which the Fc region has been separated from the sacrificial Fab;
- Figure 3 illustrates an intermediate (a) as provided by the invention and further process steps by which it may be converted into a form (c) for further reaction with another moiety; and
- Figure 4 shows an Fab Fc<sub>2</sub> product of a type which may be constructed from the form illustrated in Figure 3(c).

#### Preparation of Fc-dithiopyridine (Fc-SS-Py)

##### Principles

1. Human normal Fc gamma (see Figure 1, top right), from the predominant IgG subclass IgG1, is reduced so that its two hinge SS bonds yield four SH groups.
2. One of the Fc SH groups is used to form an SS bond with sheep Fab' gamma (see Figure 1, left), yielding the temporary species Fab-SS-Fc. Of the remaining Fc SH groups, two are now directed by SS-interchange to reform one of the original hinge SS bonds and one, directly opposite that utilised in the SS link to Fab, is blocked permanently by alkylation (see Figure 1, bottom right). Other possible reactions which may be used for blocking are discussed by Cecil and McPhee, Advances in Protein Chemistry 14:255, 1959.
3. Fab-SS-Fc is separated, by gel chromatography, from

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other products and surplus reagents in the (Fab + Fc) reaction mixture.

4. All interchain SS bonds in the Fab-SS-Fc are reduced to yield the structure of Figure 2, and the released Fab' gamma is discarded - having served its purpose of permitting the separation of Fc in which one of the four hinge SH has been blocked by alkylation.
5. A further round of SS-interchange again re-forms the available hinge SS, while the unpaired hinge SH is used to form a dithio(4-)pyridine group: (see Figure 3(a)).
6. The Fc-SS-Py is stored in this form. When required for use the S-SPy bond can be cleaved by gentle reduction at pH 4.0-4.5, and a free maleimide group (R) introduced by reaction with a large molar surplus of bismaleimide linker (e.g. o-phenylenedimaleimide), (see Figure 3(b) and 3(c). Reduction at pH 4.0-4.5 does not cleave the hinge SS. The susceptibility to reduction at acid pH shown by -SS-Py can be achieved using either 2-pyridyl or 4-pyridyl derivatives (the latter being the more susceptible), and is due to increased electrophilicity of the bond as the pyridyl N becomes protonated at the low pH (see Brockelhurst, Int. J. Biochem 10: 259, 1979).

#### Materials

Human Fc gamma is prepared from human normal IgG, itself obtained from plasma surplus to blood bank requirements, as described by Stevenson et al (Anti-Cancer Drug Design 3: 219, 1989).

Sheep IgG is prepared from serum by sequential precipitation with ammonium sulphate and DEAE-cellulose chromatography, and from it sheep F(ab' gamma)<sub>2</sub> is prepared by a standard peptic digestion (Nisonoff et al, Arch. Biochem. Biophys. 89: 230, 1960).

Dithiothreitol (DTT) and N-ethylmaleimide (NEM), both from Sigma Chemical Co., are used without further purification.



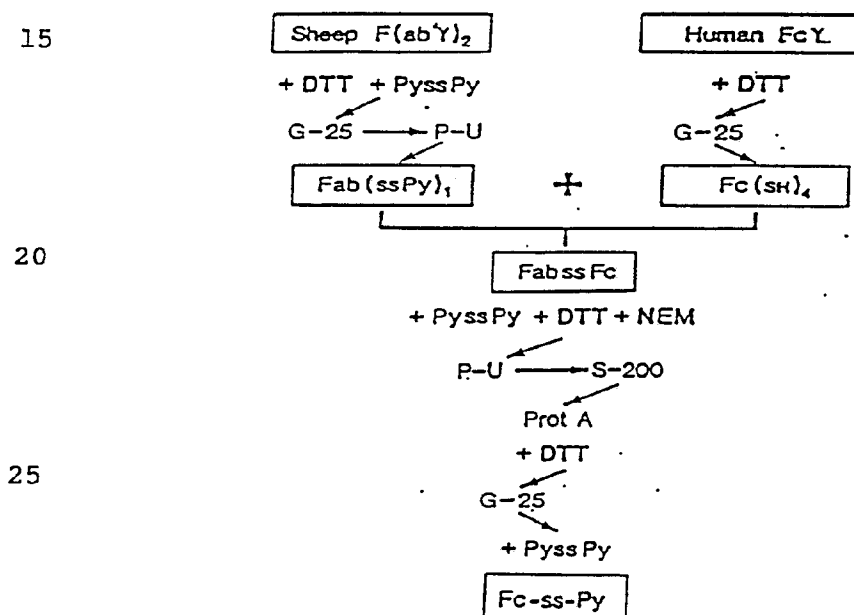
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4,4'-dipyridyl disulphide (Py-SS-Py) from Aldrich Chemical Co., is recrystallised from dimethylformamide/water.

Sephadex, Sephacryl and Protein A-Sepharose chromatography media are from Pharmacia-LKB, Phospho-Ultrogel from IBF Biotechnics.

# Details

The preparation is summarised in the flow-chart below. In this chart each "+" indicates a chemical reaction, each arrow the loading or unloading of a chromatographic column. The boxed products provide suitable subheadings for what follows.



## 30 a. Fab (SSPy)<sub>1</sub>

Sheep F(ab' gamma)<sub>2</sub>, 5mg.ml, is reduced with 1mM DTT at pH 8.0, 25°, for 30 minutes. It is then chilled and allowed to undergo SS-interchange at 5° for 15 minutes with Py-SS-Py added to 0.5mM surplus to the DTT. During this interchange the gamma-light SS bond reforms, while the SH derived from the solitary inter-gamma SS emerges as the mixed disulphide Fab-SS-Py. Passage

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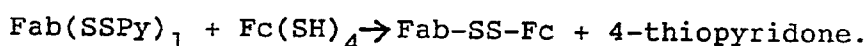
through Sephadex G-25 removes all entities of low m.w.  
( 5000) and transfers the protein to 0.03M acetate, pH  
4.5 The protein is now led onto Phospho-Ultrogel A6R  
equilibrated with the same buffer, and binds to this  
5 medium in a compact zone (about 50 mg/cm<sup>3</sup>). It is  
finally eluted from the gel at a concentration of about  
30 mg/ml with 0.5M NaCl in the same acetate buffer.

b. Fc(SH)<sub>4</sub>

Human Fc gamma at 20±2 mg/ml is reduced with 3mM DTT  
10 at pH 8.0, 25<sup>0</sup>, for 30 minutes. It is then transferred  
to 0.1M acetate, pH 4.0, with simultaneous removal of the  
DTT, by passage through Sephadex G-25.

c. Fab-SS-Fc

The Fab and Fc derivatives are mixed and incubated  
15 at 25<sup>0</sup> for 30 mins. During this period the principle  
reaction occurring is:



There is also some formation of Fab<sub>2</sub>Fc and Fab<sub>3</sub> Fc.

At the end of the incubation the mixture is diluted  
20 with 2.8 volumes of cold water and Py-SS-Py is added to  
0.17 mM. Disulphide-interchange reconstitutes those SS  
bonds in the Fc hinges where both cysteine SH remain  
free, while unpaired SH emerge as the mixed disulphide  
protein-SS-Py. The latter are reconverted to protein-SH  
25 by treatment with 0.4 mM DTT at pH 4.5. Then all further  
interchange is halted by alkylating the free SH upon  
addition of NEM to 2.9 mM.

The protein solution is now concentrated by  
absorption/desorption on Phospho-Ultrogel, as described  
30 for Fab(SSPy)<sub>1</sub>, and the concentrated solution is led  
onto Sephacryl S200 equilibrated with 0.5M NaCl, 0.1 M  
acetate, pH 5.8. The Fab-SS-Fc separates from other  
proteins and is led onto, and bound to, Protein  
A-Sepharose equilibrated with the same buffer.

35 d. Fc-SS-Py

DTT, 1mM at pH 8.0, is led through the Protein-A-  
Sepharose column, thereby releasing the Fab' gamma for

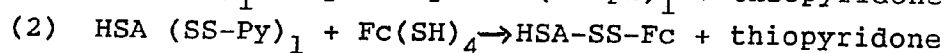
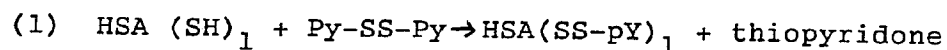
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which the column has no affinity. After washing off the Fab' gamma the Fc, in the form  $\text{Fc}(\text{SH})_3$ , is eluted with 0.1M glycine HCl, pH 3.0, and immediately transferred into 0.03 M acetate, pH 4.5, by passage through Sephadex  
5 G-25. Finally Py-SS-Py is added to 0.25 mM so that SS-interchange again restores the hinge SS and leaves the remaining non-alkylated cysteine as the mixed pyridyl disulphide (see Figure 3(a)). This intermediate has proved stable upon storage at 5°C for three months.

10 The stored intermediate may be reduced and maleimidated as illustrated in Figure 3 (b) and (c) to give Fc-mal which may be linked to any agent with a free SH group, for example,  $\text{Fab}(\text{SH})_5$  to give  $\text{FabFc}_2$  as illustrated in Figure 4.

15 In the above preparation of pyridyl Fc, sheep Fab'γ has been used to form a temporary species Fab-SS-Fc, thereby masking one SH group in the Fc hinge. At the same time the Fab-SS-Fc has a distinctive molecular weight (about 100,000), which allows it to be separated  
20 chromatographically from other reactants. These functions of sheep Fab'γ can be taken over by human serum albumin (HSA), so as to avoid additional safety precautions which might be required due to the introduction of a sheep protein. HSA has a single  
25 cysteine residue which can serve the masking function, and its molecular weight (64,500) will again endow the temporary species (HSA-SS-Fc) with a distinctive molecular weight for chromatographic separation.

Designate HSA with its single cysteine and hence its  
30 single SH group, as  $\text{HSA}(\text{SH})_1$ . Then the overall reactions required are :



However, due to the fact that the SH group on HSA is  
35 relatively unreactive with other proteins, apparently not being readily accessible on the protein surface (see Cecil & McPhee, Advances in Protein Chemistry 14: 256,

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1959), it has been found necessary to add an extension arm terminating in an SH to the cysteine residue before proceeding with reaction (1). Reactions (1) and (2) are then carried out as described above for sheep Fab'γ .

5       The preliminary steps required for HSA are as follows :

1.   HSA (Sigma Chemical Company) is first reduced at pH 7.0 with dithiothreitol (2mM) to ensure that its cysteine residue is fully in the reduced (SH) form. It is then passed through Sephadex G-25 (Pharmacia), equilibrated with .02M acetate buffer, pH 5.0 and run at 5°.
 

10
2.   The protein now reacts with o-phenylenedimaleimide (1.2 mM, pH 5.0, dimethylformamide 16% v/v) at 5° for 60 minutes, to yield HSA (mal)<sub>1</sub> with an active maleimidyl group, and is again separated from reactants of low molecular weight by passage through Sephadex.
 

15
3.   Finally the protein reacts again with dithiothreitol (1mM) to yield HSA(SH)<sub>1</sub>, with its SH group located at the end of an arm about 15 Å long.
 

20

Advantages of the process of the invention compared with previous proposals are:

- (1) The intermediate product is taken further along the road, giving a simpler and more obvious final preparative manoeuvre, and more readily suggesting further applications.
 

25
- (2) The reaction Fc(mal) + Fab(SH)<sub>5</sub> proceeds more readily without sheep Fab persisting on the Fc to cause steric hindrance.
 

30

#### Possible uses of Fc-SS-Py

The intermediates of the invention may be stored and used, when required, to attach human Fc regions to any agent with a free SH group. The agent may be selected to

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home on abnormal tissue, such as cancer or virus-infected cells, and thereby coat the tissue with Fc in a manner which will attract the destructive power of effectors (complement, macrophages, NK cells) with affinities for the Fc.

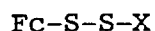
Examples of agents suitable for coupling to human Fc:-

- (1) The Fab' gamma region from a monoclonal antibody. Most antibodies readily yield this fragment upon peptic digestion. The chimeric antibody products so obtained could be used for:-
  - a. Killing cancer cells.
  - b. Suppressing host T lymphocytes in graft recipients.
  - c. Suppressing graft T lymphocytes in graft-vs-host disease.
  - d. killing virus-infected cells.
  - e. killing auto-immune lymphocytes (with antibody specific for the surface idiotypes, as for a class-specified antigen).
- (2) Any other ligand for the surface of an undesirable cell, e.g. CD4, which will attach to the viral protein gp120 displayed on the surfaces of HIV-infected cells. Such a ligand must be able to display an SH group, and this might have to be introduced by genetic or chemical engineering.

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CLAIMS

1. Intermediates for use in the preparation of derivatives of Fc regions of antibodies, the said intermediates having the general formula:



where Fc is an Fc region in which external sulphhydryl groups have been rendered substantially inert by incorporation into protein-protein disulphide bonds or by alkylation,  
and X is a protonated group causing the disulphide linkage to be susceptible to reduction at a relatively low pH.

2. Intermediates according to claim 1 characterised in that the group X is such as to permit reduction at a pH in the region of 4.
3. Intermediates according to claim 2 characterised in that the group X is pyridyl.
4. Intermediates according to any one of claims 1 to 3 characterised in that the Fc region is from human immunoglobulin.
5. A process for the production of intermediates as claimed in any one of claims 1 to 4 having the following process steps :
  - a) combining an Fc region with a "sacrificial" group;
  - b) subjecting the combination so formed to disulphide interchange and rendering potential reactive sites inert;
  - c) separating the sacrificial group from the Fc region to leave a reactive site on the Fc region; and
  - d) attaching a protonated group to the reactive site.

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6. A process according to claim 5 characterised in that the sacrificial group is selected from groups of sufficient molecular weight to enable separation of mono-substituted Fc regions from other reaction products by fractionation or the like.
7. A process according to claim 6 characterised in that the sacrificial group is an Fab' region from sheep immunoglobulin.
8. A process according to claim 6 characterised in that the sacrificial group is human serum albumin.
9. A process according to any one of claims 6 to 8 characterised in that the protonated group is pyridyl.
10. A process according to claim 9 characterised in that the pyridyl group is attached to the reactive site by reacting with dipyridyl disulphide.
11. The use of the intermediates as claimed in any one of claims 1 to 4 in a process for the preparation of derivatives of Fc regions of antibodies.
12. The use of the intermediates as claimed in any one of claims 1 to 4 in a process to attach a human Fc region to an agent with a free SH group.
13. Use according to claim 12 characterised in that the agent is selected to home on abnormal tissue.
14. Use according to claim 12 or claim 13 characterised in that the agent is the Fab' gamma region from a monoclonal antibody.
15. Use according to claim 12 or claim 13 characterised in that the agent is a ligand for the surface of an undesirable cell.

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16. Use according to any one of claims 12 to 15 in a process which includes a step wherein an intermediate is reduced.
17. Antibody derivatives obtained by use of the intermediates claimed in any one of claims 1 to 4.
18. Antibody derivatives according to claim 17 having 2 or more Fc regions.



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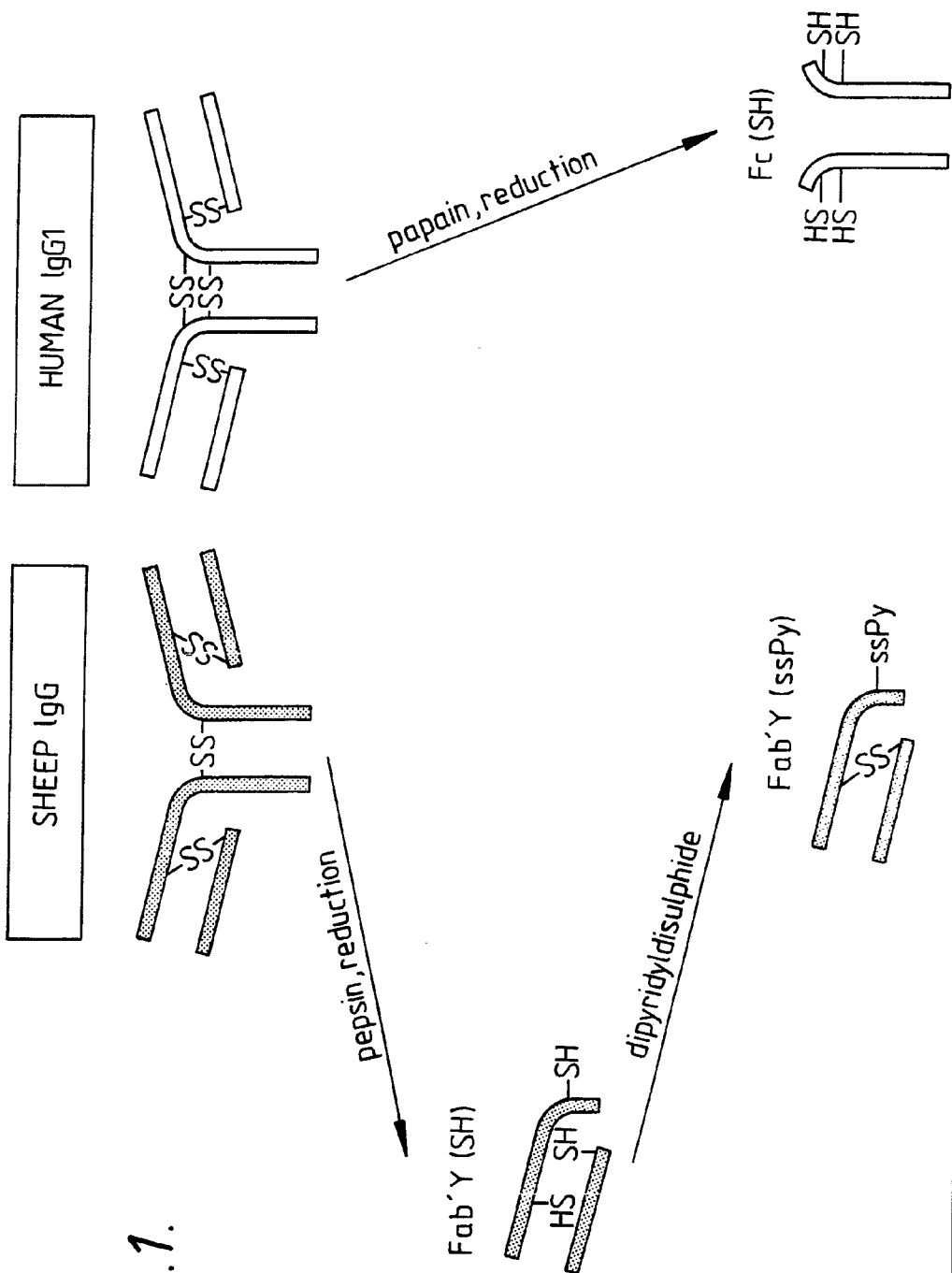
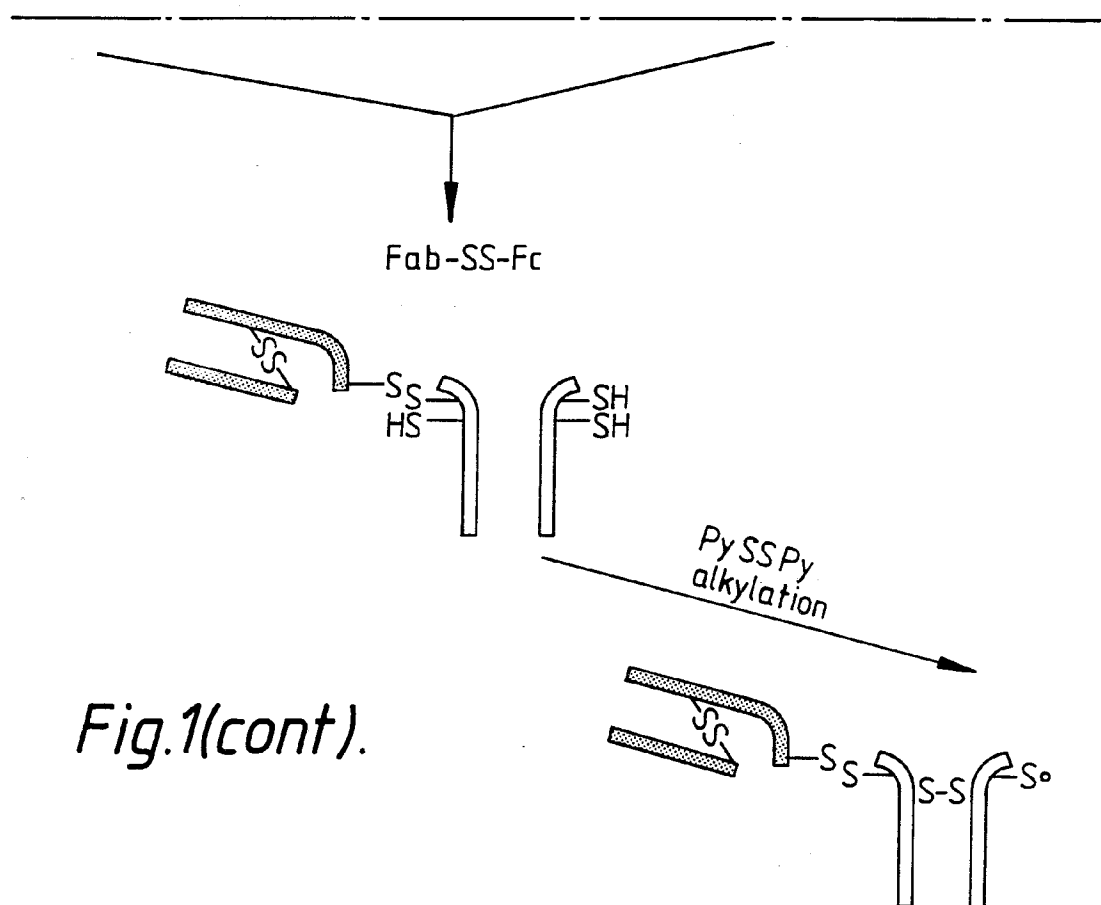


Fig.1.

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*Fig.1(cont).*

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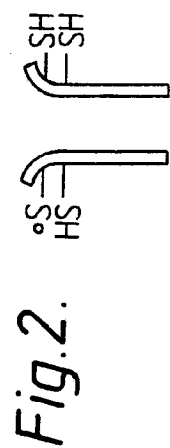
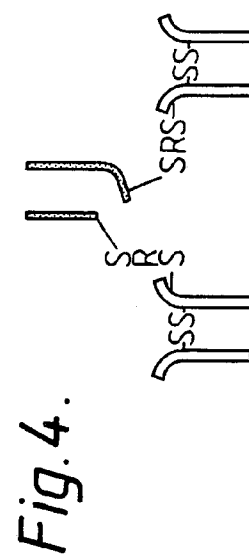
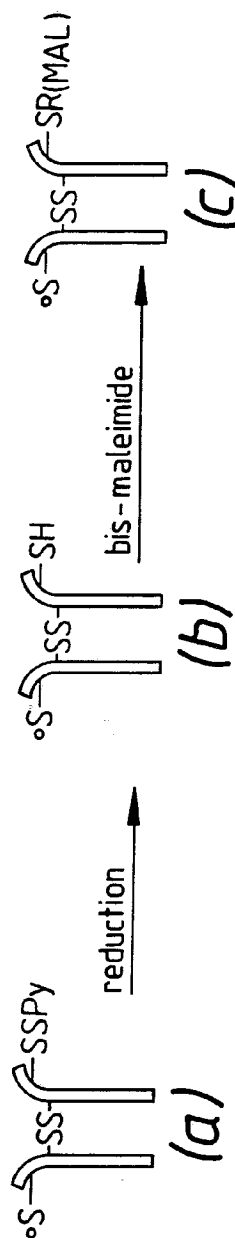


Fig. 3.

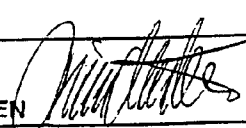


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## INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 91/00633

<b>I. CLASSIFICATION OF SUBJECT MATTER</b> (if several classification symbols apply, indicate all) <sup>4</sup>		
According to International Patent Classification (IPC) or to both National Classification and IPC		
IPC <sup>5</sup> : C 07 K 15/28, C 12 P 21/08, A 61 K 39/395		
<b>II. FIELDS SEARCHED</b>		
Minimum Documentation Searched <sup>7</sup>		
Classification System	Classification Symbols	
IPC <sup>5</sup>	C 07 K, C 12 P, A 61 K	
Documentation Searched other than Minimum Documentation to the extent that such Documents are included in the Fields Searched <sup>6</sup>		
<b>III. DOCUMENTS CONSIDERED TO BE RELEVANT<sup>8</sup></b>		
Category <sup>9</sup>	Citation of Document, <sup>10</sup> with indication, where appropriate, of the relevant passages <sup>11</sup>	Relevant to Claim No. <sup>12</sup>
P, X	WO, A1, 90/04 413 (SEARCH EXPLOITATION LIMITED) 03 May 1990 (03.05.90), see claims.	17, 18
P, A	see claims.	1-16
A	DE, A1, 3 444 765 (INSTITUT MERIEUX) 20 June 1985 (20.06.85), see claim 1.	1, 11-16
P, A	EP, A1, 0 376 770 (INSTITUT MERIEUX) 04 July 1990 (04.07.90), see claim 1.	1, 11-16
A	Chemical Abstracts, vol. 110, No. 23, issued 1989, June 5 (Columbus, Ohio, USA), Stevenson, G.T. et al. "A chimeric antibody with dual Fc regions (bisFabFc)	1-18
<p><sup>9</sup> Special categories of cited documents: <sup>10</sup></p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"Z" document member of the same patent family</p>		
<b>IV. CERTIFICATION</b>		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
25 July 1991	14. 08. 91	
International Searching Authority	Signature of Authorized Officer	
EUROPEAN PATENT OFFICE	Miss T. MORTENSEN 	

-2-

International Application No

PCT/GB 91/00633

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, " with indication, where appropriate, of the relevant passages	Relevant to Claim No.
	<p>prepared by manipulations at the IgG hinge", see pages 552-553, left and right columns, abstract-No. 210 562a, Anti-Cancer Drug Des. 1989,3(4),219-30</p> <p>-----</p>	

ANHANG

zum internationalen Recherchen-  
bericht über die internationale  
Patentanmeldung Nr.

ANNEX

to the International Search  
Report to the International Patent  
Application No.

ANNEXE

au rapport de recherche inter-  
national relatif à la demande de brevet  
international n°

PCT/8891/00633 SAE 46773

In diesem Anhang sind die Mitglieder  
der Patentfamilien der im obenge-  
nannten internationalen Recherchenbericht  
angeführten Patentedokumente angegeben.  
Diese Angaben dienen nur zur Unter-  
richtung und erfolgen ohne Gewähr.

This Annex lists the patent family  
members relating to the patent documents  
cited in the above-mentioned inter-  
national search report. The Office is  
in no way liable for these particulars  
which are given merely for the purpose  
of information.

La présente annexe indique les  
membres de la famille de brevets  
relatifs aux documents de brevets cités  
dans le rapport de recherche inter-  
national visé ci-dessus. Les renseigne-  
ments fournis sont donnés à titre indica-  
tif et n'engagent pas la responsabilité  
de l'Office.

Im Recherchenbericht angeführtes Patentedokument Patent document cited in search report Document de brevet cité dans le rapport de recherche	Datum der Veröffentlichung Publication date Date de publication	Mitglied(en) der Patentfamilie Patent family member(s) Membre(s) de la famille de brevets	Datum der Veröffentlichung Publication date Date de publication
WG-A1- 9004413	03-05-90	GB-A0- 8824869	30-11-88
DE-A1- 3444765	20-06-85	AT-E - 45498	15-09-89
		CA-A1- 1229791	01-12-87
		DE-C0- 3479398	21-09-89
		EP-A2- 147283	03-07-85
		EP-A3- 147283	14-08-85
		EP-TD- 147283	10-04-86
		EP-B1- 147283	16-08-89
		FR-A1- 2556219	14-06-85
		FR-B1- 2556219	26-06-87
		JP-A2-60185726	21-09-85
		US-A - 4719107	12-01-88
EP-A1- 376770	04-07-90	EP-TD- 376770	25-07-91
		FR-A1- 2639231	25-05-90
		JP-A2- 2231431	13-09-90